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Gebeyehu et al.

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- [54] **REAGENTS FOR INTRACELLULAR DELIVERY OF MACROMOLECULES**
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- [52] **U.S. Cl.** **514/44; 514/2**
- [58] **Field of Search** 514/2-44; 530/300, 530/350

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,897,355	1/1990	Eppstein et al.	435/240.2
4,946,787	8/1990	Eppstein et al.	435/240.2
5,049,386	9/1991	Eppstein et al.	424/427
5,171,678	12/1992	Behr et al.	435/172.3
5,208,036	5/1993	Eppstein et al.	424/450

FOREIGN PATENT DOCUMENTS

0 394 111	10/1990	European Pat. Off.	
WO 91/15501	10/1991	WIPO	
WO 91/16024	10/1991	WIPO	
WO 93/03709	3/1993	WIPO	A61K 9/127
WO 93/05162	3/1993	WIPO	C12N 15/63
WO 93/14778	8/1993	WIPO	A61K 37/00

OTHER PUBLICATIONS

- F. Barthel et al., "Gene Transfer Optimization with Lipospermine-Coated DNA", 1993, *DNA and Cell Biology* 12:6:553-560.
- J-P Behr et al., "Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA", 1989, *Proc. Natl. Acad. Sci. USA* 86:6982-6986.
- N. Düzgünes et al., "Fusion of Liposomes Containing a Novel Cationic Lipid, N-[2,3-(Dioleoyloxy)propyl]-N,N,N-trimethylammonium: Induction by Multivalent Anions and Asymmetric Fusion with Acidic Phospholipid Vesicles", 1989, *Biochemistry* 28:9179-9184.
- P.L. Felgner et al., "Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure"; 1987, *Proc. Natl. Acad. Sci. USA* 84:7413-7417.
- Gao, X. et al., "A Novel Cationic Liposome Reagent for Efficient Transfection of Mammalian Cells," (1991) *Biochim. Biophys. Res. Comm.* 179:280-285.
- Loeffler, J-P et al., "Gene Transfer into Primary and Established Mammalian Cell Lines with Lipopolyamine-Coated DNA", 1993, *Methods in Enzymology*, 217:599-618.
- Zhou, X. et al., "Lipophilic polylysines mediate efficient DNA transfection in mammalian cells", 1991, *Biochimica et Biophysica Acta*, 1065:8-14.
- Bennett, C.F. et al., "Cationic Lipids Enhance Cellular Uptake and Activity of Phosphorothioate Antisense Oligonucleotides," *Mol. Pharmacology* ((1992) 41:1023-1033.
- Bond, V.C. and Wold, B., "Poly-L-Ornithine-Mediated Transformation of Mammalian Cells," *Mol. and Cell. Biology* (Jun. 1987) 7(6):2286-2293.

- Chaney, W.G. et al., "High-Frequency Transfection of CHO Cells Using Polybrene," *Som. Cell and Mol. Genetics* (1986) 12(3):237-244.
- Dong, Y. et al., "Efficient and DNA transfection of quiescent mammalian cells using poly-L-ornithine," *Nuc. Acids Res.* (1993) 21:771-772.
- Donnelly-Roberts, D.L. and Lentz, T.L., "Structural and conformational similarity between synthetic peptides of curaremimetic neurotoxins and rabies virus glycoprotein," *Mole. Brain Res.* (1991) 11:107-113.
- Duzgunes, N. and Felgner, P., "Intracellular Delivery of Nucleic Acids and Transcription Factors by Cationic Liposomes," *Meth. in Enzymology* (1993) 221:303-317.
- Farhood, H. et al., "Effect of cationic cholesterol derivatives on gene transfer and protein kinase C activity," *Biochim. and Biophys. Acta* 1111 (1992) 239-246.
- Felgner, P.L. and Holm, M., "Cationic Liposome-Mediated Transfection," *Focus* (Spring 1989) 11(2):21-25.
- Felgner, P.L. "Cationic Lipid/Polynucleotide Condensates for In Vitro and In Vivo Polynucleotide Deliver—The Cytofectins," *J. Liposome Res.* (1993) 3(1)-3-16.
- Gao, X. and Huang, L., "Cationic Liposomes and Polymers for Gene Transfer," *J. of Liposome Res.* (1993) 3(1):17-30.
- Gao, X. and Huang, L., "Cytoplasmic expression of a reporter gene by co-delivery of T7 RNA polymerase and T7 promoter sequence with cationic liposomes," *Nuc. Acids Res.* (1993) 21(12):2867-2872.
- Huang, L. and Zhou, F., "Liposome and Immunoliposome Mediated Delivery of Proteins and Peptides," *Targeting of Drugs 3—The Challenge of Peptides and Proteins*, Gregoriadis, G. and Florence, A.T. (eds), Plenum Press, New York, NY (1992) 45-50.
- Litzinger, D.C. and Huang, L., "Amphipathic poly(ethylene glycol) 5000-stabilized dioleoylphosphatidylethanolamine liposomes accumulate in spleen," *Biochim. et Biophys. Acta* (1992) 1127:249-254.
- Litzinger, D.C. and Huang, L., "Phosphatidylethanolamine liposomes: drug delivery, gene transfer and immunodiagnostic applications," *Biochim. et Biophys. Acta* (1992) 1113:201-227.
- Nabel, G.J. and Felgner, P.L., "Direct gene transfer for immunotherapy and immunization," *Tibtech* (May 1993) 11:211-215.
- Nair, S. et al., "Class I restricted CTL recognition of a soluble protein delivered by liposomes containing lipophilic polylysines," *J. Immun. Meth.* (1992) 152:237-243.

(List continued on next page.)

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[57] **ABSTRACT**

The present invention discloses cationic lipids and lipophilic compounds useful for making lipid aggregates for delivery of macromolecules and other compounds into cells. They are especially useful for the DNA-dependent transformation of cells. Also disclosed are compositions of cationic lipids and viral components or non-viral fusagenic compounds useful for enhancing transfection.

21 Claims, No Drawings